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Surface Grafting of Poly(L-glutamates). 2. Helix Orientation

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In this paper the average helix orientation of surface-grafted poly(γ-benzyl L-glutamate) (PBLG), poly(γ-methyl L-glutamate) (PMLG), and poly(γ-methyl L-glutamate)-*co*-(γ-*n*-stearyl L-glutamate) (PMLGSLG 70/30) was investigated by means of FT-IR transmission spectroscopy. The theoretical relation between the average tilt angle (θ) and the absorption peak areas of three different backbone amide bands could be calculated because their transition dipole moment directions with respect to the helix axis were known. From the normalized absorptions, the average tilt angles of grafted helices of PBLG, PMLG, and PMLGSLG 70/30 were determined. The somewhat larger average angle of PMLG helices of 35 ± 5° with respect to the substrate compared to the value of 32 ± 5° of PBLG was due to the higher grafting density of PMLG. Because of the smaller helix diameter as a result of the smaller size of the methyl side group, more PMLG helices grew on the same surface area. Sterical hindrance and unfavorable polar interactions between unidirectional aligned helices forced the PMLG helices in a more upright arrangement. The even more perpendicular orientation of PMLGSLG 70/30 (48 ± 6°) could be the result of incorporation of mainly γ-methyl L-glutamate *N*-carboxyanhydride (MLG-NCA) monomers during the initiation step. Incorporation of the much larger γ-*n*-stearyl L-glutamate *N*-carboxyanhydride (SLG-NCA) monomers afterward lead to enlarged angles with respect to the substrate. Due to swelling, a pronounced change in helix orientation of grafted PMLGSLG 70/30 in *n*-hexadecane was observed, resulting in an almost perpendicular helix orientation.

Introduction

FT-IR transmission spectroscopy has been widely used to investigate the secondary conformations of polypeptides in general and polyglutamates in particular. Especially the backbone amide I and amide II bands of the three most frequently occurring conformations of polyglutamates, i.e., α-helix (amide I ∼ 1650 cm^{-1}; amide II ∼ 1546 cm^{-1}), β-sheet (amide I ∼ 1630 cm^{-1}; amide II ∼ 1530 cm^{-1}), and random coil (amide I ∼ 1656 cm^{-1}; amide II ∼ 1535 cm^{-1}), appear at very distinct positions in the FT-IR spectrum.[1] So, analysis of the polyglutamate chain conformation is normally very straightforward with this technique.

In addition to conformational analysis, FT-IR spectroscopy can also be used to study the orientation of α-helical polypeptides. For poly(γ-benzyl L-glutamate) (PBLG), for instance, the transition dipole moment directions of the IR absorption peaks are known[2] and the ones relevant for the work described in this paper are listed in Table 1.

Knowing that the transition dipole moment of the amide I is oriented more or less parallel to the main axis of the α-helix and that of the amide II is oriented more or less perpendicular to this axis, the ratio *D*, of the amide I over amide II absorption values, provides a rough indication of the helix orientation. The values obtained for the random situation (helices perfectly dispersed in a KBr pellet) and the parallel orientation of helices with respect to the substrate (helices in Langmuir−Blodgett (LB) films) provide the framework.

Polarized attenuated total reflection−Fourier transform infrared spectroscopy (ATR-FTIR) has been used to investigate the helix orientation of surface-grafted polyglutamate films.[3] The s-polarized light interacts with the surface-parallel components of the transition dipole moments of the vibrations, and the p-polarized light interacts with the components of the transition dipole moments in any direction. Machida et al.[3] end-grafted PBLG onto an ATR crystal and calculated an angle of 33° with respect to the substrate from the obtained spectra using the method of Sagiv.[4]

Table 1. Spectral Positions and Transition Dipole Moment Directions of Some IR Vibrations of the α-Helix Conformation of PBLG

PBLG	wavenumber, cm^{-1} transmission	transition dipole moment direction,[a] deg
amide A	3292	28
amide I	1654	39
amide II	1550	75
ester C=O	1734	53

[a] With respect to the helix axis.

The surface selection rule of reflection angle FT-IR measurements on gold,[5] due to which only transition dipole moment components perpendicular to the surface can be observed, helped researchers obtain information about the orientation of the helices in the films prepared by the self-assembly technique. These films had been obtained on gold surfaces with polymers containing strongly adsorbing thiol end groups.

Examples of this method of analysis were provided by Enriquez et al.[6,7] and Niwa et al.[8] Using the perfectly parallel orientation of the helices with respect to the substrate in LB films of PBLG, Enriquez and Samulski[7]

* To whom all correspondence should be addressed.

(1) Miyazawa, T.; Blout, E. R. *J. Am. Chem. Soc.* **1961**, *83*, 712.
(2) Tsuboi, M. *J. Polym. Sci.* **1962**, *59*, 139.
(3) Machida, S.; Urano, T. I.; Sano, K.; Kawata, Y.; Sunohara, K., Sasaki, H., Yoshiki, M., Mori, Y. *Langmuir* **1995**, *11*, 4838.
(4) Sagiv, J. *J. Am. Chem. Soc.* **1980**, *102*, 92.
(5) Greenler, R. G. *J. Chem. Phys.* **1966**, *44*, 310.
(6) Enriquez, E. P.; Gray, K. H.; Guarisco, V. F.; Linton, R. W.; Mar, K. D.; Samulski, E. T. *J. Vac. Sci. Technol. A* **1992**, *10*, 2775.

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calculated the theoretical D values for several possible orientational distribution functions of the helices in the self-assembled polymer layers and compared them with the experimental results. The two most likely helix axis distribution functions were (a) random and (b) perfect order with an angle of 37° with respect to the substrate. With IR data only, they could not distinguish between the two of them.

Niwa et al.[8] incorporated redox-active poly(L-glutamic acid) (PLGA) "guest" helices between loosely packed already grafted disulfide end capped PLGA helices. This incorporation was stimulated by macropolar interactions. As a result, they realized an increase in the average angle between the helices and the substrate surface from about 40° to 65°.

Boncheva and Vogel[9] were the first to use reference spectra of synthetic polypeptides of the two extreme helix orientations. They used LB films for the orientation of α-helices in the plane of the substrate and bacteriorhodopsin, adsorbing in a perpendicular way with respect to the substrate, as a reference for the ultimate out-of-plane orientation. The orientation of α-helices of synthetic polypeptides with intermediate orientation could be determined with reflection absorption FT-IR measurements in combination with surface plasmon resonance measurements.

For surface-grafted polypeptide films prepared with the "grafting-from" method no clear data about the helix orientation is reported. Whitesell and Chang[10,11] concluded from their RA−FT-IR measurements that a more or less perpendicular helix orientation was obtained.

Chang and Frank[12] reported values of less than 45° with respect to the substrate for their PBLG films prepared with vapor deposition.

An angle of about 30° between the helices of PBLG and the metal substrate can be evaluated from electromechanical measurements performed on surface-grafted PBLG layers between crossed aluminum electrodes, as reported by Jaworek et al.[13] The tilting of the helices accounts for the lower experimental value for the polarization compared to the theoretical one predicted for perfectly perpendicular oriented helices.

In this paper, a simple method is presented to determine the orientation of α-helical PBLG, poly(γ-methyl L-glutamate) (PMLG), and poly(γ-methyl L-glutamate)-*co*-(γ-*n*-stearyl L-glutamate) (PMLGSLG 70/30), grafted on silicon wafers by means of FT-IR transmission measurements. So, in contrast with all other work on this subject the angle of the incoming IR light beam is normal to the analyzed substrate. We used the known orientation of these polypeptides in LB films[14] (parallel to the substrate) in order to develop a semiquantitative method to reveal the orientation of the helices in surface-grafted films. We calculated the average tilt angles from the normalized areas of three different amide absorption peaks (amide A, amide I, and amide $II_{\nu\perp}$) and compared them.

Experimental Section

Materials. All synthetic procedures were carried out in a dry inert nitrogen atmosphere in specially designed glassware as described in part 1 of this series.[15] Solvents (Merck, p.a. grade) were dried on phosphorus pentoxide (P_2O_5) and distilled before use. γ-*n*-Stearyl L-glutamic acid ester (SLG) was synthesized from L-glutamic acid (Acros) and *n*-octadecanol (Acros) following the procedure of Wassermann.[16] The synthesis of the *N*-carboxyanhydrides (NCAs) from γ-benzyl (Sigma) and γ-methyl L-glutamic acid ester (Acros) and γ-*n*-stearyl L-glutamic acid ester were performed following the procedure of Dormann.[17]

Grafting Polymerization. The surface-grafting polymerizations (reaction time, 24 h) of the NCA monomers in ultradry *N*,*N*-dimethylformamide (DMF; 0.5 mol/L) were performed on silicon wafers (Topsil Semiconductor Materials A/S, Frederikssund, Denmark, both sides polished, 900 μm thickness) that had been cleaned and subsequently pretreated with (γ-aminopropyl)triethoxysilane (Acros). Silanization and polymerization were done following the procedures described in part 1 of this series.[15] The surface grafting of PMLGSLG 70/30 was performed using γ-methyl L-glutamate *N*-carboxyanhydride (MLG-NCA) and γ-*n*-stearyl L-glutamate *N*-carboxyanhydride (SLG-NCA) in the desired molar ratio.

Swelling in *n*-Hexadecane. The surface-grafted films of the random copolymer PMLGSLG 70/30 were submerged into *n*-hexadecane for 24 h at 80 °C. Just prior to analysis, the samples were blown dry with N_2.

Langmuir−Blodgett Films. PBLG (Sigma, 26.000), PMLG (Sigma, 46.000), and PMLGSLG 70/30 (synthesized following the procedure developed by Poché[18]) were dissolved in chloroform (Merck, Uvasol) at a concentration of 0.2−0.5 mg/mL. The solutions were spread on a commercially available computer controlled Lauda-Filmbalance (FW 2). The water used for the subphase was purified by a Milli-Q filtration system preceded by a reversed osmosis filtration (Elgastat spectrum SC 30). The recorded isotherms were exactly like those reported in the literature.[14,19,20] The cleaned silicon wafers were hydrophobized by treating them with a mixture of chloroform and hexamethyldisilazane (Across, 98%) (4:1 (v/v)) at 50 °C and rinsed with chloroform.

Assemblies of 10 PBLG monolayers (T = 19.1 °C; Π = 5 mN/m; *z*-transfer, 2 mm/min up- and downstroke), 14 PMLG monolayers (T = 18.3 °C; Π = 18 mN/m; *z*-transfer, 2 mm/min up- and downstroke), and 16 PMLGSLG 70/30 monolayers (T = 24.5 °C; Π = 20 mN/m; *y*-transfer, 3 mm/min up- and downstroke) were deposited onto the silicon substrates.

Infrared Measurements. FT-IR transmission measurements were performed under normal incidence with a Mattson Galaxy 6021 with a clean substrate as a reference. A set of 1000 scans with a resolution of 4 cm^{-1} was averaged.

Curve fitting of the amide A, C=O stretching of ester side chain, amide I, and amide $II_{\nu\perp}$ spectral regions were performed using the OPUS software, version 2.0 (Bruker). The amplitudes, band positions, half-widths, and Gaussian/Lorentzian compositions of the individual bands were optimized. In each case the peak areas were normalized to the peak area of the carbonyl stretch vibration of the ester side chain of the corresponding polymer in the LB film to be able to compare the spectra. From the changes in peak areas of the amide A, amide I, and amide $II_{\nu\perp}$ the helix orientation with respect to the substrate can be deduced.

Results and Discussion

In this paper a method is developed to calculate the average angle between the helices in a surface-grafted

(7) Enriquez, E. P.; Samulski, E. T. *Mater. Res. Soc. Symp. Proc.* **1992**, *255*, 423.
(8) Niwa, M.; Morikawa, M.; Higashi, N. *Angew. Chem.* **2000**, *112*, 990.
(9) Boncheva, M.; Vogel, H. *Biophys. J.* **1997**, *73*, 1056.
(10) Whitesell, J. K.; Chang, H. K. *Science* **1993**, *261*, 73.
(11) Whitesell, J. K.; Chang, H. K. *Mol. Cryst. Liq. Cryst.* **1994**, *240*, 251.
(12) Chang, Y. C.; Frank, C. W. *Langmuir* **1998**, *14*, 326.
(13) Jaworek, T.; Neher, D.; Wegner, G.; Wieringa, R. H.; Schouten, A. J. *Science* **1998**, *279*, 57.
(14) Duda, G.; Schouten, A. J.; Arndt, T.; Lieser, G.; Schmidt, G. F.; Bubeck, C.; Wegner, G. *Thin Solid Films* **1988**, *159*, 221.
(15) Wieringa, R. H.; Siesling, A. E.; Geurts, P. F. M.; Vorenkamp, E. J.; Werkman, P. J.; Erb, V.; Stamm, M.; Schouten, A. J. *Langmuir*, preceding paper in this issue (part 1).
(16) Wasserman, D.; Garber, J. D.; Meigs, F. M. U.S. Patent **1966**, 3, 285, 953.
(17) Dorman, L. C.; Shiang, W. R.; Meyers, P. A. *Synth. Commun.* **1992**, *22*, 3257.
(18) Poché, D. S.; Daly, W. H.; Russo, P. S. *Macromolecules* **1995**, *28*, 6745.
(19) Winter, C. S.; Tredgold, R. H. *Thin Solid Films* **1985**, *123*, L1.
(20) Arndt, T. Thesis, Johannes Gutenberg Universität Mainz, Germany, 1988.

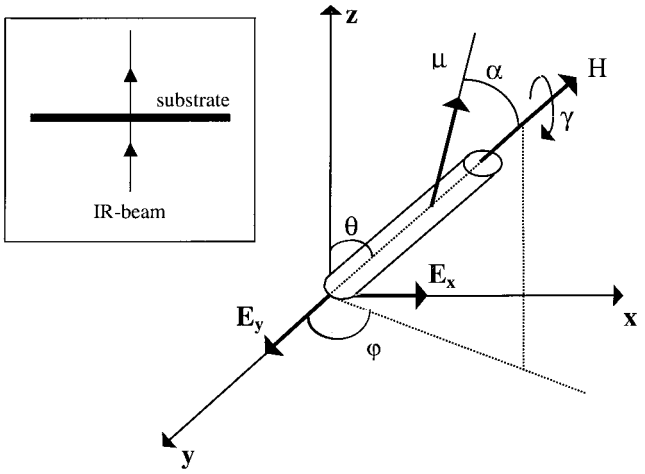

Figure 1. Helix arrangement in a laboratory frame and schematic configuration of FT-IR transmission spectroscopy measurements on a silicon wafer.

polyglutamate film and the substrate from FT-IR transmission spectra. Silicon wafers with a thickness of 1 mm that are polished on both sides are transparent for IR light and therefore make ideal substrates for the analysis with FT-IR transmission spectroscopy. When the incoming light interacts with the polyglutamate layer on the silicon support, only the electric field components $\vec{E}_x$ and $\vec{E}_y$ parallel to the substrate are active (see Figure 1). Consequently, only the active components of the vibrational transition dipole moments of the amide bonds parallel to the substrate will be detected.

To obtain an oriented state of all planar helix orientation as a starting point for the calculations, we fabricated films of α-helical polyglutamates with the Langmuir−Blodgett technique. It is known that the helices in these LB assemblies are oriented parallel to the substrate with a preferential orientation in the dipping direction of the LB process.[14,20]

For this particular orientation, the intensities of the amide A (N−H stretching) and amide I (mainly backbone C=O stretching) are the highest. Peak areas can also be used instead of the peak intensities. Since the transition dipole moments of these vibrations are oriented nearly parallel to the helix axis, there is a maximum contribution of the components parallel to the substrate. The intensity of the amide II (mainly backbone C−N stretching), which has a nearly perpendicular orientation with respect to the helix axis, is the lowest for this particular orientation. Dividing the amide I by the amide $II_{\nu\perp}$ peak intensities gives the *D* value. Therefore, the substrate-parallel orientation of the helices in LB films gives the highest *D* values. Measuring with the light **E** vector parallel to the direction of the amide I transition dipole moment even results in a nearly vanished amide $II_{\nu\perp}$ absorption.[14,20]

In Figure 1 a laboratory-fixed rectangular *x*,*y*,*z* coordinate system is shown.[21] In this figure also the IR-active electric field components $\vec{E}_x$ and $\vec{E}_y$ of the incoming light under normal incidence to the substrate (*z*-direction) are shown. The cylindrically shaped polyglutamate molecule has $\vec{H}$ as the main axis. The transition dipole moment $\vec{\mu}$ can be any amide bond studied in this report, and α is the angle between $\vec{\mu}$ and $\vec{H}$. The position of the peptide molecule is defined by the polar coordinates θ (angle between $\vec{H}$ and substrate normal) and φ (angle between the projection of $\vec{H}$ in the *x*,*y* plane of the substrate and the *y*-axis).

(21) Michl, J.; Thulstrup, E. W. *Spectroscopy with Polarized Light: Solute Alignment by Photoselection in Liquid Crystals, Polymers, and Membranes*; VCH Publishers: Deerfield Beach, FL, 1986; Chapter 4.

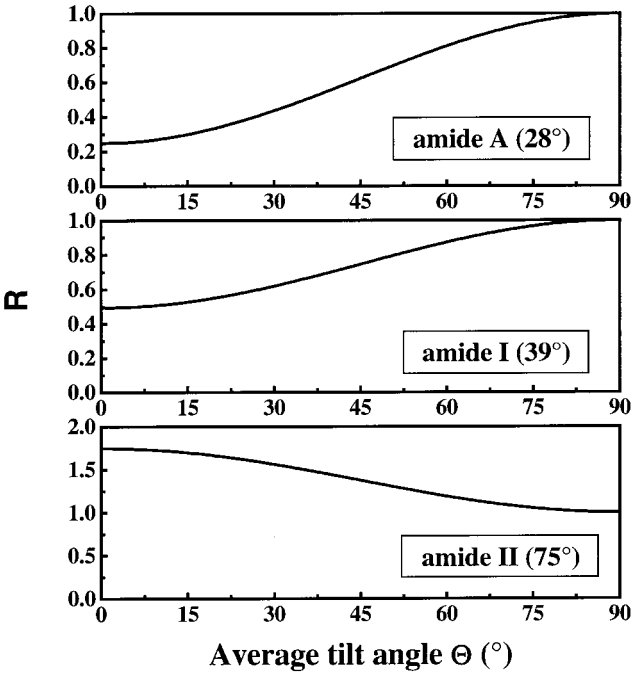

Figure 2. Relationship between absorption intensity and average helix tilt angle using the (a**, top)** amide A, (b**, middle)** amide I, and **(**c**, bottom)** amide II band, normalized to the C=O ester peak area. The number in parentheses indicates the angle between the transition dipole moment and the helix axis.[2] *R* (A_θ/A_{LB}) is the value of the peak area of the absorption of the amide vibration in the grafted film divided by the corresponding amide vibration peak area in the LB film.

In contrast to the procedure described by Samulski[7] the absorption intensity I_θ is now proportional to the square of the length of the projection of $\vec{\mu}$ on the *x*,*y* plane. This can be written as

$$I_\theta \sim \cos^2\theta \sin^2\alpha \sin^2\gamma + \sin^2\theta \cos^2\alpha + \sin^2\alpha \cos^2\gamma + 2 \cos\theta \sin\alpha \sin\gamma \sin\theta \cos\alpha \quad (1)$$

Integration over the interval $[0,2\pi]$ also averages γ out of the process and yields

$$I_\theta \sim \sin^2\theta \cos^2\alpha + {}^1/_2 \sin^2\alpha (1 + \cos^2\theta) \quad (2)$$

For an LB film ($\theta = 90°$) this results in a maximum for the first term and a minimum for the second term. The absorption intensity I_{LB} for an LB film is therefore

$$I_{LB} \sim \cos^2\alpha + {}^1/_2 \sin^2\alpha \quad (3)$$

For $\alpha = 0$ eq 2 reduces to the usual expression for the absorption intensity: $I(\theta) \sim \sin^2\alpha$.

For the evaluation of the experimental data we used the peak areas of the simulated amide bands (A_θ) instead of the peak intensities (I_θ). Both values can be used and give no significant differences. The calculated dependence of A_θ on θ and for the three different amide bonds is given in Figure 2 ($R = A_\theta/A_{LB}$). The method thus evaluates the average tilt angles of the α-helices in various surface-grafted polyglutamate films provided that there is a hemispherical (isotropic) distribution of helices with one end fixed to the substrate and that all angles with respect to the substrate are possible. Comparison of the results of the average tilt-angle calculations for the three studied

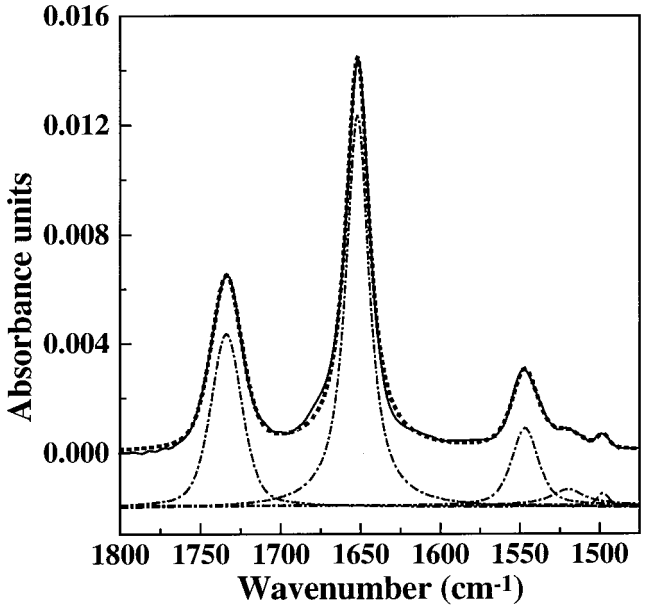

Figure 3. Curve fitting of the FT-IR transmission spectrum of the PBLG LB assembly of 10 monolayers on a silicon wafer (dotted line). The individual fitting peaks are translated vertically by −0.002 to enhance clarity.

amide vibrations should lead to a reliable average angle of the helices with respect to the substrate (90° − θ).

Poly(γ-benzyl L-glutamate). The FT-IR transmission spectrum for the region of 1475−1800 cm^{-1} of a Langmuir−Blodgett assembly of 10 monolayers of PBLG on silicon is shown in Figure 3. The dotted line represents the results of the peak-fitting procedure.

To evaluate the average orientation of the helices in surface-grafted PBLG films with respect to the substrate, the spectra of several surface-grafted PBLG layers were studied. These layers were obtained after 24 h of reaction time. The spectra were normalized to the peak area of the C=O stretching vibration of the ester side chain of the LB film. Since the side chain ester carbonyl groups are randomly distributed, the integrated area of the absorption peak of the stretching vibration is not subjected to orientation influences and can therefore be used as a measure for the amount of polymer grafted on the substrate.[14] The spectrum of the LB assembly, the spectrum of the most parallel helix orientation with respect to the substrate, and the spectrum of the most perpendicular arrangements of helices are shown in Figure 4. All other spectra obtained with several monomer batches fell between these two extreme spectra.

The normalized peak areas of the absorbances of the C=O, amide A, amide I, and amide II$_{\nu\perp}$ of the three spectra are included as the first set of data in Table 2. Calculation of D (A_I/A_{II}) provides a rough but rather good indication of the average helix orientation. For the LB film, a value of 5.35 is obtained, and the D values of 4.05−3.44 for the grafted PBLG layers indicate a more perpendicular orientation with respect to the substrate.

The D value of the grafted PBLG layer with the largest tilt angle of the helices was 3.24 before the washing procedure as opposed to 4.05 thereafter. This can also be seen by a decrease of the ester C=O stretch band after washing as reported in part 1 of this series.[15] This indicates that washing removes nongrafted helical chains from the grafted polymer layer. The free volume created by the removed chains can be filled up by the grafted ones to reduce the surface energy. These grafted helices will

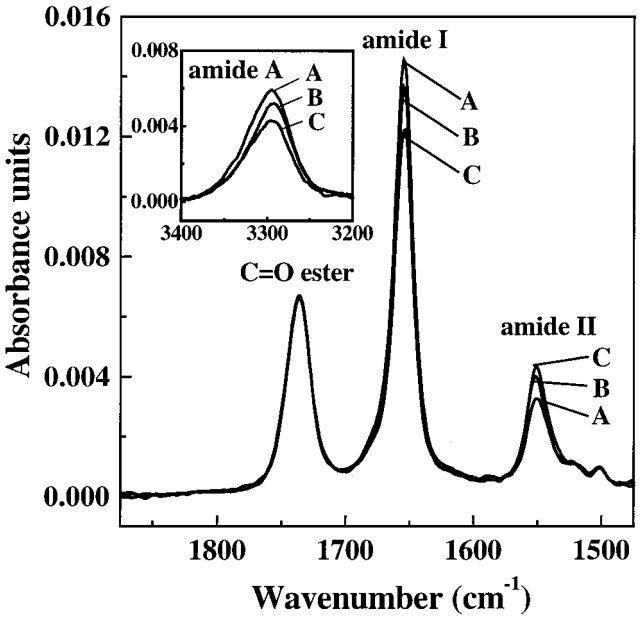

Figure 4. FT-IR transmission spectra of (A) a PBLG LB assembly of 10 monolayers, (B) the grafted PBLG layer with the smallest average angle between the helices and the substrate, and (C) the grafted PBLG layer with the largest average angle of the helices.

Table 2. Results of normalized Peak Areas, *D* (AI/AII), Tilt Angles for the PBLG LB Film and the Two Extreme Surface-Grafted Layers, and the Results for the Corresponding Films of PMLG and PMLGSLG 70/30[a]

	ester C=O	amide A	amide I (A_I)	amide II$_{\nu\perp}$ (A_{II})	D (A_I/A_{II})
		PBLG			
LB film	0.183	0.473	0.383	0.0716	5.35
film with largest θ	0.183	0.389	0.344	0.0846	4.05
film with smallest θ	0.183	0.352	0.319	0.0926	3.44
θ (deg)		62−54	64−53	63−52	
		PMLG			
LB film	0.280	0.887	0.676	0.0956	7.07
film with largest θ	0.280	0.731	0.584	0.114	5.12
film with smallest θ	0.280	0.635	0.512	0.124	4.13
θ (deg)		61−52	58−47	60−51	
		PMLGSLG 70/30			
LB film	0.286	1.037	0.724	0.0944	7.67
film with largest θ	0.286	0.712	0.564	0.128	4.41
film with smallest θ	0.286	0.545	0.474	0.143	3.31
θ (deg)		50−38	49−35	47−36	

[a] θ = angle between helix axis and substrate normal.

deviate from their unidirectional arrangement. This explains the higher D value after the washing procedure.

From eq 2 and Figure 2a−c, the average tilt angles as listed in Table 2 can be calculated. The average angle between the PBLG helices and the substrate (90° − θ) is variations found to be 32 ± 5°. These values were obtained with various monomer batches. Reactions performed with the same monomer batch gave tilt angle of 32°. The value comes close to the value that can be evaluated from the electromechanical measurements described by Jaworek et al.[13]

For the calculation of the average tilt angles, we used a model for a random distribution of helices with a certain angle between the helix axis and the substrate as suggested by Samulski.[6] This model uses perfect "rigid-rod" molecules instead of the "wormlike" nature of the PBLG helix.[22] Due to this rather flexible nature, the longer polymer chain parts, as observed in part 1 of this series,[15] have the ability to bend toward the polymer film in order

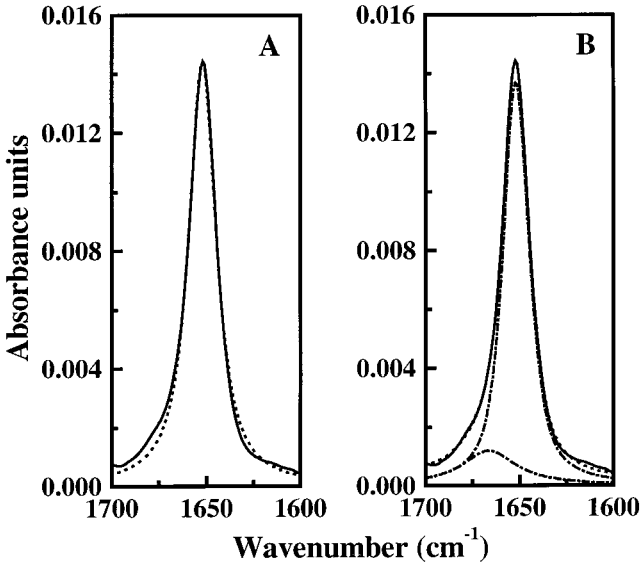

Figure 5. Peak fitting of the amide I absorption by (A) one and by (B) two peaks.

to minimize surface energy. This requires a distortion of the helices and leads to an exaggerated in-plane orientation with respect to the substrate.

The peak fitting for amide I, as shown above, is usually carried out with only one peak,[7,12,23] but assuming that there is an additional band next to the amide I band at higher wavenumbers improves the peak fit of this amide I region significantly, as can be seen in Figure 5. Distortion of the helices may contribute to this extra band at higher wavenumbers than the major amide I band. We also observed that this extra band decreased, when the average angle with respect to the substrate was higher. Apparently the effect of the distortion of helices is less at more perpendicular helix orientation.

Recently, a number of scientists showed by normal-mode analysis that nongrafted α-helical polypeptides[24] have a considerable spectral contribution on this side of the major amide I band mainly due to intermolecular transition dipole coupling between parallel oriented helices.[25,26] Because of these calculations, Boncheva and Vogel[9] fitted the amide I band with more than one peak as well. When the amide I peak is fitted with these additional peaks, the area of the major amide I peak decreases. This results in an enlarged angle with respect to the substrate that could be as much as 5°.

Now that the average angle of the helices with respect to the substrate is known, a rough estimation of the helix length, molecular weight and grafting density can be made. These results are listed in Table 3. The helix diameter for a PBLG helix is 15.5−26 Å,[23,27] depending on the orientation of the side group. The surface area per helix is therefore about 190−530 Å^2. A film thickness of 245 Å with a helix orientation of 32° with respect to the substrate means that the helices have an average length of 460 Å. Each monomer unit contributes 1.5 Å to the total helix length,[27] so the average degree of polymerization is about

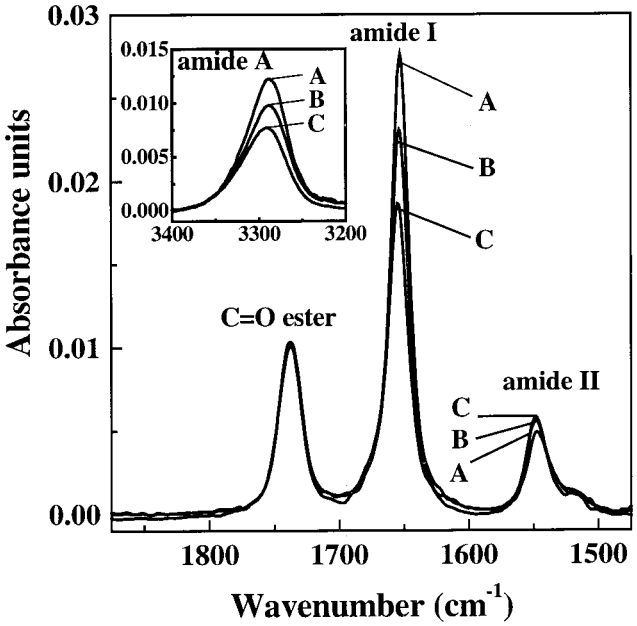

Figure 6. FT-IR transmission spectra of (A) a PMLG LB-assembly of 14 monolayers, (B) the grafted PMLG layer with the smallest average angle between the helices and the substrate, and (C) the grafted PMLG layer with the largest average angle of the helices.

Table 3. Results of the Calculations of the Average Angle between the Helices and the Substrate, Helix Lengths, Average Molecular Weights, and Grafting Densities of the Surface-Grafted PBLG, PMLG, and PMLGSLG 70/30 Films

	PBLG	PMLG	PMLGSLG 70/30
exp layer thickness (Å)	245	240	170
90-θ (deg)	32 ± 5	35 ± 5	48 ± 6
helix diam (Å)	15.5−26 [23,27]	14 [27]	17.5−37.5 [14]
theor area per helix (Å^2)	190−530	155	240−960
calc helix length (Å)	460	420	230
calc polym deg (mon units)	310	280	155
calc MW	67 500	39 900	32 700
polym dens (g/cm^3)	1.3227	1.2927	1.1129
calc area/helix (Å^2)	345	215	290
grafting dens (helices / 1000 Å^2)	≤3	≥4	>3

310. From this value an average molecular weight of 67 500 g/mol can be calculated. Using a polymer density of 1.32 g/cm,[3,27] an area per helix of 345 Å^2 can be calculated, which is somewhat smaller than the value of 465 Å^2 obtained by Oosterling et al.[28] So, the grafting density of these PBLG helices is almost 3 helices/1000 Å^2.

The same calculations can be performed for the samples produced using a monomer concentration of 2.0 mol/L and a reaction time of 1 h.[15] Indeed, higher grafting densities (275 Å^2/helix) are obtained using this higher monomer concentration.

Poly(γ-methyl L-glutamate) and Poly(γ-methyl L-glutamate)-*co*-Poly(γ-*n*-stearyl L-glutamate). The same procedure as that for the surface-grafted PBLG films was performed for the PMLG and PMLGSLG films. The corresponding spectra are shown in Figures 6 and 7, respectively. Table 2 shows that in both cases the grafted helices provide lower *D* values than the corresponding LB assemblies, indicating a more perpendicular helix orientation with respect to the substrate.

(22) Schmidt, M. *Macromolecules* **1983**, *17*, 553.
(23) Chang, Y. C.; Frank, C. W. *Langmuir* **1996**, *12*, 5824.
(24) Krimm, S.; Bandekar, J. *Adv. Protein Chem.* **1986**, *38*, 181.
(25) Krimm, S.; Reisdorf, W. C. *Faraday Discuss.* **1994**, *99*, 181.
(26) Torii, H.; Tasumi, M. In *Infrared Spectroscopy of Biomolecules*; Chapman, D., Mantsch, H. Eds.; Wiley-Liss, Inc.: New York, 1996; Chapter 1.
(27) Block, H. *Poly(γ-Benzyl-*L-Glutamate) and Other Glutamic Acid Containing Polymers; Gordon and Breach Publishers: New York, **1983,** and references cited therein.
(28) Oosterling, M. L. C. M. Thesis, University of Groningen, The Netherlands, 1994.

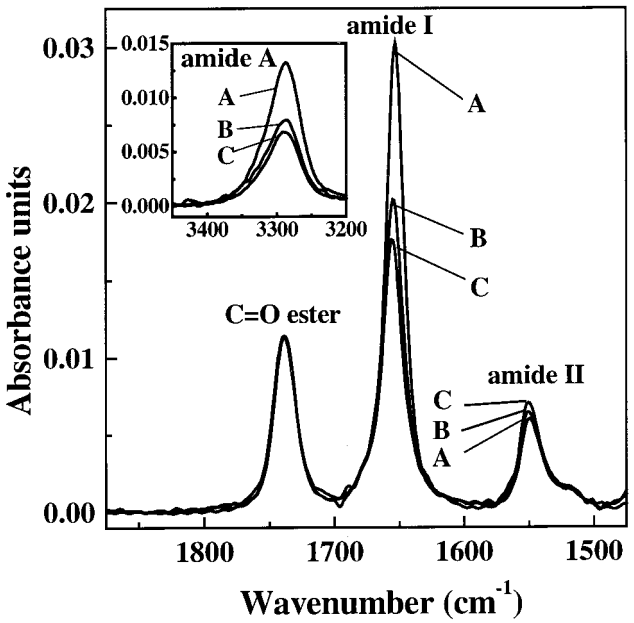

Figure 7. FT-IR transmission spectra of (A) a PMLGSLG 70/30 LB assembly of 16 monolayers, (B) the grafted PMLGSLG 70/30 layer with the smallest average angle between the helices and the substrate, and (C) the grafted PMLGSLG 70/30 layer with the largest average angle of the helices.

The results for the average orientation of the helices with respect to the substrate, the helix length, the average molecular weight, and the grafting density for both cases are summarized in Table 3. The slightly higher average angle with respect to the substrate for grafted PMLG helices compared to the PBLG value can be explained by the smaller helix diameter of PMLG of 14.0 Å instead of 15.5 Å for PBLG.[27] Consequently, a higher grafting density of more than 4 helices/1000 Å^2 can be achieved.

For the helices of the surface-grafted PMLGSLG 70/30 random copolymer an average angle with the substrate of 48 ± 6° is calculated. This higher value of the average angle with the substrate for this polymer compared to PBLG and PMLG is understandable if the helix initiation is mainly governed by the incorporation of the smaller MLG-NCA monomers. Incorporation of the much larger SLG-NCA monomers later on will therefore lead to larger angles with respect to the substrate. The helix diameter of a PMLGSLG 70/30 helix lies between 17.5 Å (totally interdigitated) and 37.5 Å (helix with stearyl chain in all trans configuration). This results in a helix area of 240−960 Å^2.[14] With an estimated polymer density of 1.11 g/cm^3[3,29] the experimentally determined area occupied by one helix is about 290 Å^2, so the grafting density is more than 3 helices/1000 Å^2.

Swelling of Surface-Grafted PMLGSLG 70/30 Polymer Layer in *n*-Hexadecane. For a stearyl content of 20−40%,[30] the interhelical amorphous alkyl regions can act as an apolar solvent for low molecular weight oleophilic molecules, like azo dyes and β-carotenoides. We tried to swell the layers in *n*-hexadecane and studied the effect on the helix orientation. The spectra before and after swelling are shown in Figure 8. The *D* value shows a dramatic change in helix orientation, dropping from 4.15 to 1.39.

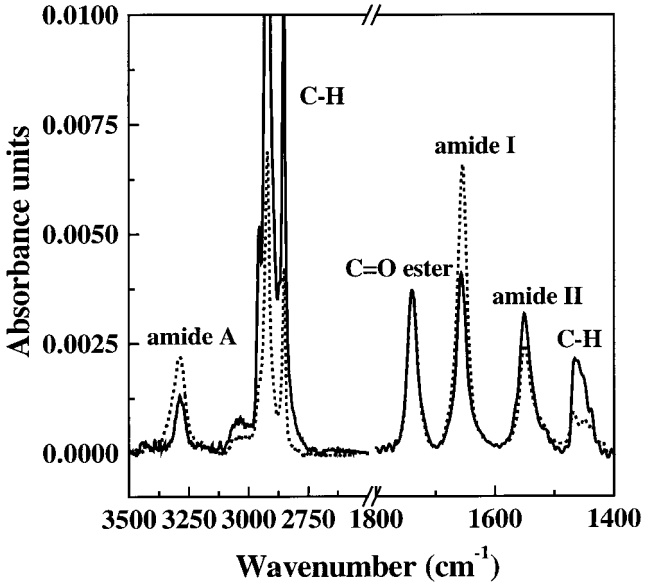

Figure 8. FT-IR transmission spectra of a grafted PMLGSLG 70/30 layer before (···) and after (—) swelling in *n*-hexadecane.

This indicates a nearly perpendicular orientation with respect to the surface. Calculating the average tilt angle values with the method described in this paper, we see a change from 44° with respect to the substrate before to about 75° after swelling. Due to swelling in *n*-hexadecane, the helices are forced to stretch away from the surface and take on a more upright orientation that is so interesting for all kinds of electrooptical applications.[31]

Conclusions

In this paper, we have evaluated the α-helix orientation of three surface-grafted polyglutamates. For the calculations, we have used the planar orientation of α-helices of the corresponding polymers in Langmuir−Blodgett films and the known angles of the transition dipole moment directions of three studied amide vibrations (amide A, amide I, and amide II$_{\nu\perp}$).

The rough indications about the helix orientation provided by the *D* values agree very well with the calculated tilt angles. The changes in average angles with the substrate of PBLG, PMLG, and PMLGSLG 70/30 helices can be explained by changes in grafting density mainly caused by the size of the monomer. In the case of copolymers, this average angle might be determined by the size of the first incorporated monomer. At higher conversions, the average orientation of the helices is more parallel to the substrate, since longer chain parts can bend toward the polypeptide surface due to van der Waals interactions.

Swelling of grafted PMLGSLG 70/30 layers results in a nearly perpendicular orientation with respect to the substrate.

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(29) Kasuya, S.; Sasaki, S.; Watanabe, J.; Fukuda, Y.; Uematsu, I. *Polym. Bull.* **1982**, *7*, 241.

(30) Duda, G.; Wegner, G. *Makromol. Chem. Rapid. Commun.* **1988**, *9*, 495.

(31) Whitesell, J. K.; Chang, H. K.; Fox, M. A.; Galoppini, E.; Watkins, D. M.; Fox, H.; Hong, B. *Pure Appl. Chem.* **1996**, *68*, 1469.